WHAT IS CLAIMED IS:

1. A biologically active transfer vector incorporating no viral genes comprising, linked:

(a) a 5' long terminal repeat (LTR) sequence derived from a VL30 retrotransposon comprising a transcription initiation site for RNA;

(b) an encapsidation sequence positioned 3' of the 5' LTR;

(c) a primer binding site sequence derived from a VL30 retrotransposon and positioned 3' of the 5' LTR;

(d) a 3' LTR sequence derived from a VL30 retrotransposon positioned 3' of the primer binding site which includes:

(1) sequences necessary for polyadenylation of a RNA transcript initiated in the 5' LTR;

(2) sequences necessary for reverse transcription of the RNA transcript from step (d)(1) into a double stranded cDNA;

(e) a polypurine tract sequence from a VL30 retrotransposon located 5' to the 3' LTR; and

(f) sequences within each LTR which are necessary for integration of the biologically active transfer vector into the genome of a recipient cell,

wherein the vector sequences comprise no more than 2 kbp.

2. The vector of claim 1 wherein the encapsidation sequence is derived from a VL30 retrotransposon.

3. The vector of claim 1 wherein the VL30 retrotransposon sequences are derived from a mouse VL30 retrotransposon.

4. The vector of claim 1 selected from the group consisting of VLP, VLPB, VLPP, VLPPB, VLCN, VLDN, VLPBN, VLPBNS, VLPPBN,

VLPPBNS, VLSN, VLPSNO, VLATGSAF, VLBEN, VLPPBGZ, VLIL2EN, VLATGF, VLATGR, VLOVBGH, VLSVP, VLATGSAR and VL30-MVM.

5. The vector of claim 1 wherein the homology of the vector sequences to murine leukemia virus viral helper sequences is reduced so that the likelihood of recombination between the vector and murine leukemia virus is decreased or eliminated.

6. The vector of claim 1 further comprising at least one ATG codon, positioned 3' of the transcription initiation site for RNA located in the 5' LTR, followed by a short open reading frame so that RNA transcripts initiated in the 5' LTR and terminated within the 3' LTR of the vector are more efficiently packaged into virions than the RNA transcripts are translated by ribosomes.

7. The vector of claim 1 further comprising sequences recognized as splicing signals, positioned 3' of an initiation site for RNA transcripts located in the 5' LTR, so that RNA transcripts initiated in the 5' LTR and terminated within the 3' LTR of the vector are more efficiently translated by ribosomes than the RNA transcripts are packaged into virions.

8. The vector of claim 1 wherein the 3' LTR further comprises a transcriptional unit.

9. The vector of claim 8 wherein the transcriptional unit is a VL30-derived transcriptional unit.

10. The vector of claim 8 wherein the transcriptional unit is derived by amplifying DNA or messenger RNA sequences encoding a preselected transcriptional unit using oligonucleotides which contain at least a

portion of the preselected transcriptional unit.

11. The vector of claim 1 further comprising at least one DNA sequence encoding a protein, an autonomously replicating element, or a RNA sequence positioned 3' of the transcription initiation site in the 5' LTR.

12. The vector of claim 11 wherein the autonomously replicating element is a DNA transposon.

13. The vector of claim 11 wherein the autonomously replicating element is a virus.

14. The vector of claim 11 wherein the DNA sequence encodes a toxin.

15. The vector of claim 14 wherein the DNA sequence encoding the toxin comprises two exons.

16. The vector of claim 15 wherein exon 1 of the toxin gene is inserted within the 3' LTR of the vector and is operably linked to a transcriptional unit, and exon 2 is inserted 3' of the 5' LTR of the vector and 5' to the 3' LTR of the vector such that the cDNA derived from the vector encodes exon 1 then exon 2.

17. The vector of claim 11 wherein the DNA sequence encodes a reporter gene or a selectable marker gene.

18. The vector of claim 11 wherein the DNA sequence is followed by a polyd(T) tract.

19. The vector of claim 11 wherein the DNA sequence is operably linked to an internal transcriptional unit.

20. The vector of claim 19 wherein the internal transcriptional unit confers tissue-specific transcription, hormone-specific transcription, or developmental-specific transcription.

21. The vector of claim 19 wherein the internal transcriptional unit is derived by amplifying DNA or messenger RNA sequences encoding a preselected transcriptional unit using oligonucleotides which contain at least a portion of the preselected transcriptional unit.

22. The vector of claim 19 wherein the internal transcriptional unit is a VL30-derived transcriptional unit.

23. A method of producing recipient cells augmented with an exogenous double-stranded DNA comprising:

(a) packaging the vector of claim 1 or RNA transcribed from the vector by encapsulating the vector or RNA transcribed from the vector in a lipid carrier particle together with primers and enzymes necessary for reverse transcription, integration, or both, to yield a packaged vector or vector-derived RNA;

(b) exposing the packaged vector or vector-derived RNA to recipient cells so that the material is taken up by the recipient cells;

(c) forming double-stranded DNA from the vector or vector-derived RNA in the recipient cells; and

(d) identifying or isolating recipient cells containing the double-stranded DNA.

24. A method of producing double-stranded cDNA derived from a transfer vector in a recipient cell comprising:

(a) introducing the vector of claim 1 into a donor cell to yield a transformed donor cell;

(b) transcribing RNA from the vector in the transformed donor

cell to yield vector derived RNA;

(c) packaging the vector derived RNA into a virion to yield a virus comprising vector-derived RNA;

(d) exposing a recipient cell to the virus so that the virus is taken up by the recipient cell;

(e) reverse transcribing vector-derived RNA from the virus to form double-stranded cDNA in the recipient cell; and

(f) identifying or isolating progeny of the recipient cell containing the double-stranded cDNA.

25. The method of claim 24 in which the donor cell is selected from the group consisting of psi2 and PA317.

26. The method of claim 24 wherein the double-stranded cDNA is integrated into the genome of the recipient cell.

27. The method of claim 26 wherein the chromosomal location of the integrated form of the double-stranded cDNA in a recipient cell is visualized by means of *in situ* hybridization.

28. The method of claim 26 further comprising cloning the genomic DNA sequences of the recipient cell flanking the integrated cDNA into a bacteriophage or plasmid vector and the cloned flanking sequences are expressed as RNA or protein in cultured cells.

29. The method of claim 27 wherein the nucleotide sequence of the flanking DNA sequences are determined by nucleotide sequencing methods.

30. The method of claim 24 wherein the recipient cell is infected with a retrovirus or a retrovirus-derived vector.

31. The method of claim 30 wherein the double-stranded cDNA in the recipient cell is transcribed so that the RNA derived from the double-stranded cDNA is packaged more efficiently than RNA derived from the retrovirus or the retroviral derived vector.

32. A method of transferring a DNA sequence into an animal comprising:

(a) inserting a preselected DNA sequence into the vector of claim 1 3' of the transcription initiation site in the 5' LTR to yield a DNA transfer vector;

(b) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;

(c) transcribing RNA from the DNA transfer vector in the transformed donor cell to yield a transfer vector RNA;

(d) packaging the transfer vector RNA to yield a virion with the transfer vector RNA;

(e) infecting a recipient cell with the virion;

(f) reverse transcribing the transfer vector RNA packaged in the virion in the recipient cell to yield a cDNA;

(g) identifying progeny cells of the recipient cell containing the cDNA; and

(h) introducing the progeny cells of step (g) into an organ, a tissue, an embryo, or an animal host.

33. The method of claim 32 wherein the donor cell is selected from the group consisting of psi2 and PA317.

34. The method of claim 32 wherein the recipient cell is an embryonic stem cell, a pluripotent stem cell, or an embryo.

35. The method of claim 32 wherein the recipient cell is a bone marrow cell which is first treated with mycophenolic acid to render it quiescent, and then treated with a cytokine to induce proliferation

during the infection of the bone marrow cell with the virion.

36. A method of transferring a DNA sequence into an animal comprising:
(a) inserting a preselected DNA sequence into the vector of claim 1 3' of the transcription initiation site in the 5' LTR to yield a DNA transfer vector;
(b) introducing the DNA transfer vector into a donor cell capable of packaging nucleic acid molecules into a virion to yield a transformed donor cell;
(c) introducing the transformed donor cell, or progeny of the transformed donor cell into an organ, a tissue, an embryo, or an animal host, and
(d) identifying a cell within the animal in which a virion, comprising vector-derived RNA, produced by the transformed donor cell, or progeny cells of the transformed donor cell, has entered the animal cell, RNA has been reverse transcribed, and a resulting cDNA integrated into the genome of the cell.

37. The method of claim 36 wherein the donor cell is selected from the group consisting of psi2 and PA317.

38. A method of introducing and expressing a DNA sequence in an oviduct or embryo of egg laying species comprising:
(a) inserting a DNA sequence encoding a protein or RNA into a vector to yield a DNA transfer vector wherein the DNA sequence is operably linked to a transcription unit in the DNA transfer vector;
(b) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;
(c) transcribing RNA from the DNA transfer vector in the transformed donor cell to yield RNA derived from the DNA transfer vector;

(d) packaging the RNA derived from the DNA transfer vector into a virion to yield a virus comprising RNA derived from the DNA transfer vector;

(e) introducing the virus into the oviduct or germ line embryo of egg laying species; and

(f) identifying animals which express the protein or RNA encoded by the DNA sequence.

39. The method of claim 38 wherein the DNA sequence is operably linked toa chicken ovalbumin gene promoter and a signal peptide.

40. The method of claim 38 wherein the DNA sequence is inserted into the vector of claim 1 3' of the transcription initiation site in the 5' LTR.

41. A method of introducing and expressing a DNA sequence in an oviduct or embryo of egg laying species comprising:

(a) inserting a DNA sequence encoding a protein or RNA into a vector to yield a DNA transfer vector wherein the DNA sequence is operably linked to a transcription unit in the DNA transfer vector;

(b) introducing the DNA transfer vector into a donor cell to yield a donor cell transformed with the DNA transfer vector;

(c) introducing the transformed donor cell, or progeny of the transformed donor cell, into the oviduct or germ line embryo of egg laying species; and

(d) identifying animals which express the protein or RNA encoded by the DNA sequence.

42. The method of claim 41 wherein the DNA sequence is operably linked to a chicken ovalbumin gene promoter and a signal peptide.

43. The method of claim 41 wherein the DNA sequence is inserted into the vector of claim 1 3' of the transcription initiation site in the 5' LTR.

44. The method of claim 41 wherein the transcription unit is selected from the group consisting of an ovotransferrin transcription unit, an ovomucoid transcription unit, an ovalbumin transcription unit, a lysozyme transcription unit and an avidin transcription unit.

45. A method of inserting and expressing a DNA sequence in a mammary cell comprising;

(a) operably linking a DNA sequence encoding a protein to a transcriptional promoter containing a hormone inducible transcription element and a mammary cell specific transcription element to yield an expression vector;

(b) inserting the expression vector into the vector of claim 1 3' of the transcription initiation site in the 5' LTR to yield a DNA transfer vector;

(c) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;

(d) transcribing RNA from the DNA transfer vector in the donor cell to yield a transfer vector RNA;

(e) packaging the transfer vector RNA into a virion to yield a virus with transfer vector RNA;

(f) introducing the virus into a mammary cell to yield a transformed mammary cell; and

(g) producing a protein encoded by the DNA sequence in the transformed mammary cell.

46. The method of claim 45 wherein the mammary-cell specific transcription element is selected from the group consisting of a casein promoter, a whey acidic protein promoter, and a lactoferrin promoter.

47. The method of claim 45 wherein the transformed mammary cell is present in a mammary gland of a nonhuman mammal.

48. The method of claim 45 wherein exposing the transformed mammary cell to a hormone results in a change in the level of protein encoded by the DNA sequence.

49. A method of producing a double-stranded cDNA containing a gene which is capable of homologous recombination with a DNA sequence present in the genome of a recipient cell, comprising:

(a) linking a polyd(T) tract to the 3' end of the gene to yield a hybrid gene;

(b) introducing the hybrid gene into the vector of claim 1 3' of the transcription initiation site in 5' LTR to yield a DNA transfer vector;

(c) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;

(d) transcribing RNA from the DNA transfer vector in the transformed donor cell to yield a transfer vector RNA;

(e) packaging the transfer vector RNA into a viral particle to yield a virus;

(f) exposing a recipient cell to the virus so that the virus is taken up by the recipient cell to yield a transformed recipient cell;

(g) reverse transcribing the transfer vector RNA from the virus in the transformed recipient cell to yield a double-stranded cDNA derived from the hybrid gene; and

(h) identifying or isolating progeny cells of the transformed recipient cell which contain an integrated form of the double-stranded cDNA.

50. A method of expressing a toxin gene encoded by more than one exon

in a recipient cell but not in a donor cell as a result of the structural rearrangement of the exons during viral replication comprising:

(a) inserting exon 1 of the toxin gene into the 3' LTR of the vector of claim 1 wherein exon 1 is operably linked to a transcription unit to yield a hybrid vector;

(b) introducing the remaining exons of the toxin gene into the hybrid vector to yield a DNA transfer vector;

(c) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;

(d) transcribing RNA from the DNA transfer vector in the transformed donor cell to yield a transfer vector RNA;

(e) packaging the transfer vector RNA into a viral particle to yield a virus;

(f) exposing a recipient cell to the virus so that the virus is taken up by the recipient cell to yield a transformed recipient cell;

(g) reverse transcribing the transfer vector RNA from the virus in the transformed recipient cell to yield a double-stranded cDNA; and

(h) expressing the toxin from the double-stranded DNA in the transformed recipient cell.

51. A method for increasing the titer of viral stocks by introducing a DNA sequence into a biologically active transfer vector to effect the equilibrium between the packaging and translation of viral RNA comprising:

(a) introducing a DNA sequence into the vector of claim 1 3' of a transcription initiation site in the 5' LTR to yield a DNA transfer vector;

(b) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;

(c) transcribing RNA from the DNA transfer vector in the

transformed donor cell to yield a transfer vector RNA;
(d) packaging the transfer vector RNA into a viral particle to yield virions;
(e) collecting the virions produced in step (d) to yield a virus stock; and
(f) determining the titer of virus stock relative to a virus stock produced from the vector of claim 1.

52. The method of claim 51 wherein the DNA sequence comprises a splice acceptor site, a splice donor site, or both.

53. The method of claim 51 wherein the DNA sequence comprises at least one stop codon in the same reading frame as an ATG codon wherein the ATG codon is separated form the stop codon by no more than 70 base pairs and the stop codon is 3' to the ATG.

54. A method of delivering an autonomously replicating DNA sequence to the genome of a recipient cell comprising:
(a) introducing a preselected autonomously replicating DNA sequence into the vector of claim 1 3' of the transcription initiation site in the 5' LTR to yield a DNA transfer vector;
(b) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;
(c) transcribing RNA from the DNA transfer vector in the transformed donor cell to yield a transfer vector RNA;
(d) packaging the transfer vector RNA into a viral particle to yield a virus;
(e) exposing a recipient cell to the virus so that the virus is taken up by the recipient cell to yield a transformed recipient cell;
(f) reverse transcribing the transfer vector RNA from the virus in the transformed recipient cell to yield at least a single-

stranded cDNA derived from the transfer vector RNA; and
(g) identifying or isolating progeny of the transformed recipient cell which contain the cDNA.

55. The method of claim 54 wherein the DNA sequence is a DNA transposon.

56. The method of claim 54 wherein the DNA sequence is an autonomously replicating virus.

57. A method of rescuing a transcriptional unit from a cell comprising:
(a) amplifying DNA or messenger RNA sequences encoding a preselected transcriptional unit using oligonucleotides which contain at least a portion of the preselected transcriptional unit to yield a transcriptional unit cassette; and
(b) inserting the transcriptional unit cassette into the vector of claim 1.

58. The method of claim 57 wherein the transcriptional unit cassette is inserted into the 3' LTR of the vector of claim 1 5' of the sequences necessary for reverse transcription and polyadenylation.

59. The method of claim 57 wherein the transcriptional unit cassette is inserted 3' of the transcription initiation site in the 5' LTR.

60. The method of claim 57 wherein the transcriptional unit is a long terminal repeat.

61. A method for reconstituting blood with genetically modified hematopoietic stem cells derived from bone marrow comprising:
(a) inserting a preselected DNA sequence into a LTR-containing vector 3' of the transcription initiation site in the 5'

LTR to yield a DNA transfer vector;

(b) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;

(c) transcribing RNA from the DNA transfer vector in the transformed donor cell to yield a transfer vector RNA;

(d) packaging the transfer vector RNA to yield a virion with the transfer vector RNA; and

(e) infecting bone marrow cells which were first treated with an agent to render the cells quiescent, and then treated with at least one cytokine to induce the proliferation of the cells with the virion.

62. The method of claim 61 wherein the LTR-containing vector is a retroviral vector.

63. The method of claim 61 wherein the LTR-containing vector is the vector of claim 1.

64. The method of claim 61 further comprising introducing the infected bone marrow cells into an animal.

65. The method of claim 64 wherein the animal was subjected to irradiation or other means for ablating the endogenous bone marrow of the animal prior to the introduction of the infected bone marrow.

66. The method of claim 61 wherein the cytokine is selected from the group consisting of tumor necrosis factor-alpha, leukemia inhibitor factor, interleukin-1, interleukin-3, interleukin-6 and Steel factor.

67. A method for reconstituting blood with genetically modified hematopoietic stem cells derived from bone marrow comprising:

(a) inserting a preselected DNA sequence into the vector of

claim 1 3' of the transcription initiation site in the 5' LTR to yield a DNA transfer vector;

(b) introducing the DNA transfer vector into a donor cell capable of packaging nucleic acid molecules into a virion to yield a transformed donor cell;

(c) introducing the transformed donor cell, or progeny of the transformed donor cell into an organ, a tissue, an embryo, or an animal host, and

(d) identifying a bone marrow cell within the animal in which a virion, comprising vector-derived RNA, produced by the transformed donor cell, or progeny cells of the transformed donor cell, has entered the animal bone marrow cell, virion RNA has been reverse transcribed, and a resulting cDNA integrated into the genome of the bone marrow cell.

68. A method of reconstituting tissues with genetically modified embryonic stem cells comprising:

(a) inserting a preselected DNA sequence into a VL30-derived vector 3' of the transcription initiation site in the 5' LTR to yield a DNA transfer vector;

(b) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;

(c) transcribing RNA from the DNA transfer vector in the transformed donor cell to yield a transfer vector RNA;

(d) packaging the transfer vector RNA to yield virions with the transfer vector RNA;

(e) infecting an embryonic stem cell with the virions;

(f) reverse transcribing the transfer vector RNA packaged in the virion in the embryonic stem cell to yield a cDNA; and

(g) identifying embryonic stem cells containing the cDNA and culturing the embryonic stem cells containing the cDNA to permit their maturation.

69. The method of claim 68 wherein the VL30-derived vector is the vector of claim 1.

70. The method of claim 68 further comprising introducing the matured embryonic stem cells containing the cDNA into an organ, a tissue, an embryo, or an animal host.

71. The method of claim 68 wherein the embryonic stem cell has been modified with respect to the histocompatibility antigens present on the stem cell surface.

72. A method of reconstituting tissues with genetically modified embryonic stem cells comprising:

(a) inserting a preselected DNA sequence into a VL30-derived vector 3' of the transcription initiation site in the 5' LTR to yield a DNA transfer vector;

(b) introducing the DNA transfer vector into a donor cell to yield a transformed donor cell;

(c) introducing the transformed donor cell, or progeny of the transformed donor cell into an organ, a tissue, an embryo, or an animal host, and

(d) identifying an embryonic stem cell, or progeny thereof, within the animal in which a virion, comprising vector-derived RNA, produced by the transformed donor cell, or progeny cells of the transformed donor cell, has entered the embryonic stem cell, virion RNA has been reverse transcribed, and a resulting cDNA integrated into the genome of the embryonic stem cell.

73. The method of claim 72 wherein the VL30-derived vector is the vector of claim 1.

74. A method of increasing the resistance of a cell to infection by a

retrovirus comprising introducing at least one retrotransposon-derived vector that is transcribed at a high efficiency into the cell so that RNA transcribed from the retrotransposon vector outcompetes RNA derived from the retrovirus for packaging proteins or cellular translational machinery, following infection by the retrovirus.

75. A method of selecting retrotransposon-derived vectors which replicate efficiently comprising:

(a) introducing a retrotransposon-derived transfer vector into a donor helper cell to yield a transformed donor helper cell;

(b) transcribing RNA from the DNA transfer vector in the donor helper cell to yield transfer vector RNA;

(c) packaging the transfer vector RNA with packaging proteins provided by the helper cell into a virion to yield a virus with transfer vector RNA;

(d) infecting a different donor helper cell with the virus;

(e) reverse transcribing transfer vector RNA in the donor helper cell of step (d) to yield a cDNA;

(f) transcribing RNA from the cDNA to yield transfer vector RNA;

(g) packaging transfer vector RNA of step (f) with packaging proteins provided by the helper cell of step (d) into a virion to yield a virus with transfer vector RNA; and

(h) repeating steps (d)-(g) until the vector which predominates is one which replicates more efficiently than a vector which was not repeatedly passaged through helper cells.

76. The method of claim 75 wherein the DNA transfer vector is the vector of claim 1.

ABSTRACT

VECTORS FOR GENE TRANSFER

Improved recombinant retrotransposon vectors for gene transfer are disclosed. The synthetic vectors are truncated so as to reduce or altogether eliminate homologous recombination with retroviral helper sequences found in helper cells used to propagate the vectors, making them safer for use in humans and providing more space for therapeutic genes. The vectors transmit foreign DNA efficiently, are stable, enable abundant RNA expression from the retrotransposon transcriptional promoter, and through their diversity permit many useful applications in therapeutics and transgenics. Methods are described for rescuing tissue-specifics promoters obtaining expression in primary cells, mapping the genome and other techniques of therapeutic and transgenic utility.